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☐ 1: J Biol Buccale. 1990 Dec;18(4):299-306.

Histochemical localization of transforming growth factor-beta 1 in developing rat molars using antibodies to different epitopes.

D'Souza RN, Happonen RP, Flanders KC, Butler WT.

Department of Anatomical Sciences, University of Texas Health Science Center, Houston 77225.

In this study transforming growth factor-beta 1 (TGF-beta 1) has been immunolocalized in developing rat molars using two well characterized polyclonal antibodies, Anti-CC and Anti-LC, that recognize extracellular and intracellular TGF-beta 1, respectively. With immunohistochemical methods and the ABC-peroxidase system of detection, the growth factor was immunolocalized within the ectodermally derived enamel organ and the neural crest-derived dental papilla at the early and advanced bell stages of development. With Anti-CC, widespread and abundant extracellular TGF-beta 1 was found associated with the stellate reticulum and within central and apical regions of dental papilla mesenchyme. In contrast, Anti-LC localized TGF-beta 1 intensely within the cells of the outer dental epithelium. Moderate immunostaining for TGF-beta 1 with Anti-LC was also evident within the apical cytoplasm of inner dental epithelial cells and odontoblasts. These findings support the hypothesis that TGF-beta 1 may play a paracrine role in tooth development by regulating the epithelial-mesenchymal interactions that influence growth and cytodifferentiation events.

PMID: 1710211 [PubMed - indexed for MEDLINE]

Jun 21 2006 12:14:26

□ 1: Biochemistry. 1988 Jan 26;27(2):739-46.

Related Articles, Links

Antibodies to peptide determinants in transforming growth factor beta and their applications.

Flanders KC, Roberts AB, Ling N, Fleurdelys BE, Sporn MB.

Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892.

Polyclonal antibodies have been raised to a series of synthetic peptides which correspond to essentially all regions of the transforming growth factor beta 1 (TGF-beta 1) molecule. All antisera were evaluated for their abilities to react with TGF-beta 1 and TGF-beta 2 in either the native or reduced form in enzyme-linked immunosorbent assays, Western blots, and immunoprecipitation assays. While all antisera demonstrated some ability to recognize TGF-beta 1 in these systems, there was limited cross-reactivity with TGF-beta 2, suggesting that substantial sequence or conformational differences exist between the two growth factors. On Western blots 5-10 ng of purified human platelet TGF-beta 1 could be detected when probed with affinity-purified peptide antisera generated against peptides corresponding to residues 48-77, 50-75, and 78-109 of the 112 amino acid TGF-beta 1 monomer. Antisera raised against peptides 50-75 and 78-109 were most effective in immunoprecipitating reduced and native 125I-TGF-beta 1, respectively. The antisera also were tested for their effectiveness in blocking the binding of 125I-TGF-beta 1 to its receptor. Anti-peptide 78-109 and anti-peptide 50-75 blocked 80% and 40% of the binding, respectively, while antibodies against amino-terminal peptides were without effect. These data suggest that the carboxyl-terminal region of TGF-beta 1 may play a significant role in the binding of the native ligand to its receptor.

PMID: 2450577 [PubMed - indexed for MEDLINE]

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File: PGPB

Oct 2, 2003

DOCUMENT-IDENTIFIER: US 20030185898 A1

TITLE: Cartilage-Derived morphogenetic proteins

Detail Description Paragraph:

[0038] Thus, cloned inserts having novel BMP-like sequences were isolated, radiolabeled and used to screen both human and bovine articular cartilage cDNA libraries. Six clones were isolated from the human cDNA library. The sizes of the EcoRI inserts (2.1 kb) and their restriction maps were found to be identical for all six clones. One clone was used for nucleotide sequencing. An open reading frame encoding a BMP related protein, designated CDMP-1, was identified. It appeared that the human cDNA clone lacked the coding region for the first methionine and signal peptide. The 5' end of the human CDMP-1 was subsequently obtained from a human genomic clone isolated from a library constructed in the EMBL-3 vector (Clontech, Palo Alto, Calif.). The 5' end of human CDMP-1 contained a consensus translation initiation sequence disclosed by Kozak (J. Biol. Chem. 266:19867 (1991)) immediately followed by a putative transmembrane signal sequence described by Von Heijne (Nucl. Acids Res. 14:4683 (1986)). The nucleotide sequence and the translation of the open reading frame of CDMP-1 are presented in FIG. 1. As shown in the figure, the CDMP-1 protein was predicted to have 500 amino acids, to consist of a pro-region of 376 amino acids, a typical cleavage site (Arg-Xaa-Xaa-Arg/Ala) (SEQ ID NO:9), and a C-terminal domain of 120 amino acids containing the seven highly conserved cysteines characteristic of the TGF-.beta. gene family. A single N-linked glycosylation site is located in the pro-region (marked by an asterisk in the figure). A putative signal peptide is underlined in bold. A termination codon (TGA) is shown in the 5' untranslated region. The bold dashed underline indicates the fragment obtained by RT-PCR that was subsequently used to screen cDNA libraries. The 13 amino acid peptide used to raise polyclonal antibodies in rabbits is underlined. A vertical arrowhead marks the boundary between the sequence obtained from genomic DNA and cDNA.

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TCAGAACGA GTTATTTTCA GCTGCTGACT GGAGAGGCTG CAGCTCTGGA TACGAGACGA TTTCCTACTAT GGGACTGGAT ACNACACAC ACCGGGCAGA CTTCAGAGAT TTACAGACTGA
GGAGAAACCC TTTCCTCTCT GCTGCTACTG CTGCTGCCGC TGCTTTTGAAG AGTCACCTTC CTTCCTATGGT TTTTCTCTGC AACCCAGAGG CACTTCTGCT GCTGCCGCTG TTCTCTTTGG
TTTCTATTACG CGGCTGGCCA GAGGATGAGA CTCCCCAAAC TCTCTACTTT CTTCCTTTGG TACTGCTTT GACTGGAOCT GGATTATATC TGGACTGTGT TGGGTGCCCC TGACTTTGGG 32
CAGAGACCCC AGCGGTCCAG GCGAGATTG GCCAAGCAG AGGCCAAGGA GAGGCCCCC CTGCGCCGGA AGCTCTTCAG GCCAGGGGGT CACAGCTATG GTGGGGGGGC CACCAATGCC
Q R P Q G S R P G L A K A E A K E R P P L A R N V F R P G G H S Y G G A T N A 72
ANITCTAAGG C'AAAGGAGG CACCGGGGAG ACAGAGGCC TGACAGAGCC CAAGAGGAT GAACCCAAAA AGCTGCCCCC CAGACCGGGC GGCCTCTAAG CCNAGCCAGG ACACCTCTCC
N A R A K G G T G Q T G G L T Q P K K D E P K K L P P R P G P E P K P G H P P 112
CAACAGGC AGCTACAGC CCGACTGTG ACCCCAAAG GACAGCTTCC CGAGAGCAAG GCACCCCAA AGCAGGATC TGTCCCCAGC TGTCTCTCTG TCAAGAGGC CAGCGAGCCC
O T R Q A T A R T V T P K G Q L P G K A P K A G S V P S S F L L K K A R E P 152
GGCTCCAC GAGAGCCAA GGAGCGTTT CCGCCACCC CCATCACCC CACAGGTAC ATGCTCTGC TGTACAGAC GCTGTCCGAT GCTACAGAA AGGAGGCCAA CAGCAGCTG
G P P R E P K E P F R P P P I T P H E Y M L S L Y R T L S D A D R K G N S S V 192
ANITCTAAGG C'AGACTTCC CACACCATC ACCAGCTTTA TTGACAAAG GCAAGTAC CAGGTCCG TGGTACGAA GCAGAGTAC GTTTTGA CA TTAGTCCCT GAGAGAGAT
K L E A G L A N T I T S F I D K G Q D D R G P V V R K Q R Y V F D I S A L E K D 232
GGCTTCTG GCGCGAGCT CGGATCTTG CGAAGAGC CCTCGACAC GGCACGCA CGGTCGCC CAGGTCCCC GCGTCCAG CTGAAGCTGT CCAGCTGCC CAGCGCGCG
G L L G A E L R I L R K K P S D T A K P A V P R S R R A A Q L K L S S C P S G R 272
CAGCTCCG CTTTCTGGA TGTGCTCC GTCCAGGCC TGGTGGATC TGGTCTGACA TCTGGAAGCT CTTCGGAAC TTTAAGAACT CGGCTCAGT GTCCTCGAG
Q P A A L L D V R S V P G L D G S G W E V F D I W K L F R N F K N S A Q L C L E 312
CTGAGTCT CGAGACGGG CAGGACCGT GACCTCGTG GCTCGGCTT CGACCGCC GCGCGCAGG TCCAGGAA GGCCTGTGTC CTGTTGTGTTG GCGCCACCA GAAAGCGGAC
L E A W E R G R T V D L R G L G F D R A A R Q V H E K A L F L V F G R T K K R D 352
CTGTTCTTTA ATGAGATTAA GGCCTGCTT GCGCAGGAG ATAGACCT GTATGATAC CTGTTGAGC AGCGCGGAA AGCGCGG CCAATCGGCA CTGCGCAGG CAGCGAGCC
L F F N E I K A R S G Q D D K T V Y E Y L F S Q R R K R R A P S A T R Q G K R P 392
ACCAAGACC TTAGGCTCG CTGAGTCGG AAGCAGTCC ATGTCACTT CAAGACATG GCGTGGAG ACTGATCAT CCGACCTT CAGTACAGG CTTCCTACTG CGAGCGGCTG
S K N L K A R C S R K A L H V N F K D M G W D D W I L A P L E Y E A F H C E G L 412
TGGAGTCC CATTCGCT CCACTGGAG CCCAGATC ATGAGTCT CACAGACCTG ATGAACTGGA TGGACCCGGA GTCCACACCA CCACTCTCT GTGTGCCAC CCGGCTAGT
C E F P L R S H L E P T N H A V I D T L M N S M D P E S T P P T C C V P T R L S 472
CCCAACGA TCTCTTCTAT TGACTCTGCC AACAGGTGG TGTATAGCA GTATAGGAC ATGCTGTGG AGTGTGTGG CTGAGGTAG CAGCAGTGC CTTCTGTCTT CTGCTGTGC 501
P I S I L F I D S A N N V V Y K Q Y E D M V V E S C G C R . (SEQ. ID NO.: 13)
ACATCCAG AGCCCTTCC TGCCTCTG GNATCAGA GGGTCAGA ACTGTGCA GAGCTCTA CACAGTGG TGAAGGAT CAATAGCTT CCTGCTCTC TGAGTGTAC
TTGGCTAA GCGCCCTTT TATCCAGAG TTGCTCTGG TGAGATTC TGGCTCTG CTGATGAC CAGTGGAG CAGGTCCA CACAGTCCA CTCTGAATGG GACTGTGAC
CAGGAACAG TGCTTTCCGA TGAGACTAG CCGACATTT CTCTCACT GCGCTCTC AGCTCTGCA CTCTCTAAG CACTCTCAG GAGGCCACA GGTGCCACTG CTTCTCMA
TCACATTTGT GCTGTGAC TTCTGTCC TGGACATG GAGACTGA CTGGCAGA GTGGCAGA AGAGAGAG GCTTGGATAG AGTTGAGGAG TGTGAGGCTG TTAGACTGTT
AGATTAAT GTATTATGAT GAGTAATAA GCAAACTGT CCTTAATAA AAAAAAAAA A (SEQ. ID NO.: 11)

FIGURE 1

TGF- β Subgroup Pattern

	Cys	Cys	Val	Arg	Pro	Leu	Tyr	Ile	Asp	Phe	Arg	Xaa	Asp	Leu	Gly	Trp
	1				5					10					15	
5	Lys	Trp	Ile	His	Glu	Pro	Lys	Gly	Tyr	Xaa	Ala	Asn	Phe	Cys	Xaa	Gly
				20					25					30		
	Xaa	Cys	Pro	Tyr	Xaa	Trp	Ser	Xaa	Asp	Thr	Gln	Xaa	Ser	Xaa	Val	Leu
			35					40					45			
10	Xaa	Leu	Tyr	Asn	Xaa	Xaa	Asn	Pro	Xaa	Ala	Ser	Ala	Xaa	Pro	Cys	Cys
		50					55					60				
	Val	Pro	Gln	Xaa	Leu	Glu	Pro	Leu	Xaa	Ile	Xaa	Tyr	Tyr	Val	Gly	Arg
15	65					70				75					80	
	Xaa	Xaa	Lys	Val	Glu	Gln	Leu	Ser	Asn	Met	Xaa	Val	Xaa	Ser	Cys	Lys
				85						90					95	
20	Cys	Ser														

Each Xaa can be independently selected from a group of one or more specified amino acids defined as follows, wherein: Xaa12 is Arg or Lys; Xaa26 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa31 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa33 is Ala, Gly, Pro, Ser, or Thr; Xaa37 is Ile, Leu, Met or Val; Xaa40 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa44 is His, Phe, Trp or Tyr; Xaa46 is Arg or Lys; Xaa49 is Ala, Gly, Pro, Ser, or Thr; Xaa53 is Arg, Asn, Asp, Gln, Glu, His, Lys, Ser or Thr; Xaa54 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa57 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa61 is Ala, Gly, Pro, Ser, or Thr; Xaa68 is Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr or Val; Xaa73 is Ala, Gly, Pro, Ser, or Thr; Xaa75 is Ile, Leu, Met or Val; Xaa81 is Arg, Asn, Asp, Gln, Glu, His, Lys, Ser or Thr; Xaa82 is Ala, Gly, Pro, Ser, or Thr; Xaa91 is Ile or Val; Xaa93 is Arg or Lys.

The Vg/dpp subgroup pattern, SEQ ID NO: 65, accommodates the homologies shared among members of the Vg/dpp subgroup identified to date including dpp, vg-1,

TGF-BETA SUPERFAMILY MUTANT MEMBERS, INCLUDING MORPHOGENIC PROTEINS

This application incorporates herein by reference the entire disclosure of co-
pending utility applications U.S.S.N. 09/375,333 and 09/374,958 (Attorney Docket
5 Nos. STK-075 and STK-076) filed on even date herewith.

Field of the Invention

The invention relates to modified proteins, and DNAs encoding the same,
which are biosynthetic, chemosynthetic or recombinant constructs derived from the
TGF- β superfamily of structurally-related proteins, including modified morphogenic
10 proteins.

Background of the Invention

The TGF- β superfamily includes five distinct forms of TGF- β (Sporn and
Roberts (1990) in Peptide Growth Factors and Their Receptors, Sporn and Roberts,
eds., Springer-Verlag: Berlin pp. 419-472), as well as the differentiation factors Vg-1
15 (Weeks and Melton (1987) Cell 51: 861-867), DPP-C polypeptide (Padgett et al.
(1987) Nature 325: 81-84), the hormones activin and inhibin (Mason et al. (1985)
Nature 318: 659-663; Mason et al. (1987) Growth Factors 1: 77-88), the Mullerian-
inhibiting substance, MIS (Cate et al. (1986) Cell 45: 685-698), osteogenic and
morphogenic proteins OP-1 (PCT/US90/05903), OP-2 (PCT/US91/07654), OP-3
20 (PCT/WO94/10202), the BMPs, (see U.S. Patent Nos. 4,877,864; 5,141,905;
5,013,649; 5,116,738; 5,108,922; 5,106,748; and 5,155,058), the developmentally
regulated protein Vgr-1 (Lyons et al. (1989) Proc. Natl. Acad. Sci. USA 86: 4554-
4558) and the growth/differentiation factors GDF-1, GDF-3, GDF-9 and dorsalin-1

	TGF- β 3	42	Ten Dijke <i>et al.</i> (1988) <u>Proc. Natl. Acad. Sci. USA</u> 85:4715-4719; Derynck <i>et al.</i> (1988) <u>EMBO J.</u> 7:3737-3743.
5	TGF- β 4	43	Burt <i>et al.</i> (1992) <u>Mol. Endocrinol.</u> 6:989-922.
	TGF- β 5	44	Kondaiah <i>et al.</i> (1990) <u>J. Biol. Chem</u> 265:1089-1093
10	dpp	45	Padgett <i>et al.</i> (1987) <u>Nature</u> 325:81-84; Paganiban <i>et al.</i> (1990) <u>Mol. Cell Biol.</u> 10:2669-2677.
	vg-1	46	Weeks <i>et al.</i> (1987) <u>Cell</u> 51:861-867
15	vgr-1	47	Lyons <i>et al.</i> (1989) <u>Proc. Natl. Acad. Sci USA</u> 86:4554-4558
20	60A	48	Wharton <i>et al.</i> (1991) <u>Proc. Natl. Acad. Sci. USA</u> 88:9214-9218; Doctor <i>et al.</i> (1992) <u>Dev. Biol.</u> 151:491-505
	BMP-2A	49	Wozney <i>et al.</i> (1988) <u>Science</u> 242: 1528-1534
25	BMP-3	50	Wozney <i>et al.</i> (1988) <u>Science</u> 242: 1528-1534
	BMP-4	51	Wozney <i>et al.</i> (1988) <u>Science</u> 242: 1528-1534
30	BMP-5	52	Celeste <i>et al.</i> (1990) <u>Proc. Natl. Acad. Sci. USA</u> 87: 9843-9847
	BMP-6	53	Celeste <i>et al.</i> (1990) <u>Proc. Natl. Acad. Sci. USA</u> 87: 9843-9847
35	Dorsalin	54	Basler <i>et al.</i> (1993) <u>Cell</u> 73:687-702
	OP-1	55	Ozkaynak <i>et al.</i> (1990) <u>Embo. J.</u> 9:2085-2093; Celeste <i>et al.</i> (1990) <u>Proc. Natl. Acad. Sci. USA</u> 87: 9843-9847
40	OP-2	56	Ozkaynak <i>et al.</i> (1992) <u>J. Biol. Chem</u> 267: 25220-25227
	OP-3	57	Ozkaynak <i>et al.</i> PCT/WO94/10203 SEQ ID NO: 1
45	GDF-1	58	Lee (1990) <u>Mol. Endocrinol.</u> 4: 1034-1040
	GDF-3	59	McPherron <i>et al.</i> (1993) <u>J. Biol. Chem.</u> 268:3444-3449

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<input type="checkbox"/>	L2	L1 near50 (cterminal or c-terminal or carboxy-terminal or carboxyterminal or carboxyl-terminal or carboxylterminal or 351-366 or 353-366)	332
<input type="checkbox"/>	L3	L2 same (epitope or antibody or antibodies or monoclonal or mono-clonal or scfv or fab or polyclonal or poly-clonal or antisera or antiserum)	30
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Oct 27, 2005

DOCUMENT-IDENTIFIER: US 20050239134 A1

TITLE: Combinatorial selection of phosphorothioate single-stranded DNA aptamers for TGF-beta-1 protein

Summary of Invention Paragraph:

[0008] Whereas both antisense phosphorothioate oligonucleotides and gene therapy expressing antisense unmodified oligonucleotides modulate TGF-.beta. activity by blocking gene expression of the TGF-.beta. protein, alternative approaches in which TGF-.beta. activity is modulated following TGF-.beta. expression are also under study. Design studies on synthetic peptide antagonists to the TGF-.beta. cell surface receptors have shown that linear peptide analogs of the amino acids 83-112 C-terminal binding region of the TGF-.beta. ligand failed to bind to T.beta. receptors, whereas the introduction of a disulfide bridge so as to constrain conformationally the peptides to a configuration similar to that of the native configuration of TGF-.beta.1 and TGF-.beta.2, yielded peptide binding to the non-signaling co-receptor T.beta.REII and to the extracellular matrix protein/ligand trap decorin, which is known to bind to and inhibit activity of TGF-.beta.. Thus, the constrained peptide may act on two signaling pathway steps to reduce TGF-.beta. signaling. The peptides that were constrained conformationally failed to bind to the signaling T.beta. RII receptor (Roswell Park Cancer Institute website, 2003).

Summary of Invention Paragraph:

[0027] TGF-.beta.2 has a 10-fold greater binding affinity for the binding protein (ligand trap) .alpha.2-macroglobulin than does TGF-.beta.1 (Burmester, et al., 1993) and also binds with higher affinity to a glycosyl phosphatidylinositol (GPI)-linked binding protein that is expressed on the surface of vascular endothelial cells (Qian, et al., 1999). TGF-.beta.1, but not TGF-.beta.2, binds to endoglin, a cell surface protein abundant in endothelial cells (Qian, et al., 1999). At least three different functional domains of the TGF-.beta. molecule have been shown, in these studies, to be modulators of TGF-.beta. interaction with binding proteins- amino acids 40-68 domain modulating interaction with endoglin, amino acids 92-98 domain modulating interaction with GPI-linked binding protein and amino acids 4047 domain modulating interaction with .alpha.-2 macroglobulin.

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